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SCIENTIFIC NOTE

Efficacy and toxicity of insecticides to green peach aphid

Eficácia e toxicidade de inseticidas para o pulgão verde do pessegueiro

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ABSTRACT: Outbreaks of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) populations are not rare in *Brassica* crops in Pernambuco State (Brazil). The aim of the present study was to perform laboratory assessments in seven insecticide formulations against *M. persicae*. The direct spray and leaf dip methods were used in the experiment. *M. persicae* mortality rates were subjected to variance and Probit analyses. Based on the results, thiamethoxam + lambda-cyhalothrin, thiamethoxam, lambda-cyhalothrin and thiamethoxam + chlorantraniliprole were the most efficient insecticides after 24-hour exposure, since they caused over 95% mortality through the direct spray method. LC_{50s} ranged from 0.10 to 9.1 mg a.i./L in chlorfenapyr and chlorantraniliprole, respectively, in the leaf dip method. All insecticides were effective against *M. persicae,* except for chlorantraniliprole. Chlorfenapyr, lambda-cyhalothrin, pymetrozine and thiamethoxam + lambda-cyhalothrin were the most toxic to this aphid.

RESUMO: *Surtos populacionais de Myzus persicae (Sulzer) (Hemiptera: Aphididae) não são raros em cultivos de Brassica no Estado de Pernambuco (Brasil). O objetivo desse estudo foi realizar avaliação laboratorial de sete formulações de inseticidas contra M. persicae. Para tanto, foram utilizados o método de pulverização direta e o método de imersão de folha. A mortalidade de M. persicae foi submetida à análise de variância e análise de Probit. Os resultados mostraram que após 24 horas de exposição, tiametoxam + lambda-cialotrina, tiametoxam, lambda-cialotrina e tiametoxam + clorantraniliprole foram mais eficientes, causando mortalidade acima de 95% pelo método de pulverização direta. Utilizando o método de imersão de folha, as* CL_{50s} *foram de 0,10 até 9,1 mg i.a/L para clorfenapir e clorantraniliprole, respectivamente. Com exceção ao clorantraniliprole, todos os inseticidas foram eficazes para M. persicae sendo que clorfenapir, lambda-cyhalothrin, pimetrozina e tiametoxam + lambda-cialotrina foram mais tóxicos para o afídio.*

PALAVRAS-CHAVE
Myzus persicae
Piretroide
Neonicotinoide
Diamidas antranílicas
Suscetibilidade

KEYWORDS
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1 Introduction

Green peach or peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is among groups of pest-insects that have successfully exploited the agricultural environment and acknowledged worldwide as one of the most harming agricultural pests (Margaritopoulos et al., 2009). *M. persicae* is a sucking insect able to transmit more than 100 viruses and to use over 400 plants from 50 different families (Schoonhoven et al., 2005). Outbreaks of *M. persicae* populations in brassica crops often account for losses faced by producers from Pernambuco State (Northeastern Brazil). *M. persicae*, *Brevicoryne brassicae* (L.) and *Lipaphis erysimi* (Kalt.) are the most harming pest aphids in Brassica crops, since they cause up to 70%-80% yield reduction under favorable conditions (Khan et al., 2015)

High density *M. persicae* populations attacked *Brassica rapa* var. *pekinensis* (known in Brazil as "*couve-chinesa*") at the experimental field from the Universidade Federal de Pernambuco (UFRPE). The *M. persicae* population in this site does not have history of exposure to any insecticide type; therefore, it is possible assuming that this population was susceptible to new insecticides such as chlorantraniliprole, chlorfenapyr and pymetrozine in *Brassica rapa* var. *Pekinensis* crops at UFRPE. There is no register of any insecticide to control *M. persicae* in *B. rapa* var. *Pekinensis* (Brasil, 2003)

Chemical control is the main method to mitigate damages caused by *M. persicae*; but the insecticides has put strong selection pressure on *M. persicae* populations leading to evolution of insecticide-resistance (Bass et al., 2014). Nowadays, the Arthropod Pesticide Resistance Database (APRD) makes available 469 cases (80 compounds) of insecticide resistance in *M. persicae*; this species was preceded by *Tetranychus urticae* Koch (Acari: Tetranychidae) (517 cases) and *Plutella xylostella* L. (Lepidoptera: Plutellidae) (866 cases) and in the rank of resistance to insecticides.

Studies about the efficacy and toxicity of novel insecticides are important to help managing pest aphids, mainly in crops with few registers pesticides to chemical-control. Chlorantraniliprole, pymetrozine and chlorfenapyr are part of novel groups of insecticides, namely: anthranilic diamides, pyridine azomethine and pyrrole analog, respectively. Chlorantraniliprole activates insect ryanodine receptors and lead to fast muscle dysfunction and paralysis (Cordova et al., 2006). This process takes place due to depletion in calcium reserves, which are essential for muscle contraction (Cordova et al., 2006).

The exposure to pymetrozine causes immediate and irreversible feeding cessation, but not because of the deterrent action. Actually, its mode of action results from stylet penetration blockage (Kayser et al., 1994), but this compounds' target mechanism has not yet been identified. Chlorfenapyr is a pro-insecticide and its oxidative removal from the N-ethoxymethyl group by mixed function oxidases leads it to its toxic form, which uncouples oxidative phosphorylation in mitochondria. Consequently, ATP production is disrupted and followed by energy loss, which leads to cell disjunction and, eventually, to the death of exposed organisms (Raghavendra et al., 2011). Two more compounds were included in the present study, namely: thiamethoxam (neonicotinoid) and lambda-cyhalothrin (pyrethroid). Thiamethoxam (neonicotinoid) is a nicotinic receptor agonists of acetylcholine and lambda-cyhalothrin (pyrethroid) is a sodium channel modulator. Thiamethoxam bonds account for the persistent activation of nicotinic receptors and both compounds thiamethoxam (neonicotinoid) and lambda-cyhalothrin (pyrethroid) cause super excitation of the nervous system.

The aim of the present study was to assess the efficacy and toxicity of chlorantraniliprole, chlorfenapyr, thiamethoxam, lambda-cyhalothrin, pymetrozine, thiamethoxam + chlorantraniliprole, and thiamethoxam + lambda-cyhalothrin to *M. persicae* in *Brassica rapa* var. *pekinensis.*

2 Materials and Methods

The *M. persicae* colony was collected at the *Brassica rapa* var. *pekinensis* crop in the production site from the Universidade Federal Rural de Pernambuco (UFRPE) in July, 2015 (Pernambuco State - Brazil). The site has no history of exposure to any insecticide. Leaves with natural *M. persicae* infestation were taken to the Laboratory of Interaction Insect-Toxic (LIIT).

Two experiments were carried out in the present study: (i) direct spray and (ii) leaf dip methods. Thiamethoxam (14.1%) + lambda-cyhalothrin (10.6%) (Neonicotinoid + Pyrethroid, Engeo Pleno, Syngenta S.A.), lambda-cyhalothrin (Karate 50 EC, Syngenta S.A.), chlorfenapyr (Pyrrole, Pirate 2 40 SC, Basf), thiamethoxam (Actara 250 WG, Syngenta S.A.), chlorantraniliprole (Anthranilic Diamide, Premio SC, Dupont), thiamethoxam (20%) + chlorantraniliprole (10%) (Voliam Flexi, Syngenta S.A.) and pymetrozine (Pyridine Azomethine, Chess 500 WG, Syngenta S.A.) were the insecticides used in the experiment.

2.1 Direct spray method

B. rapa var. *Pekinensis* leaves with natural *M. persicae* infestation were cut into 5.0-cm diameter discs for the direct spray method. In total, 20 insects were kept on each disc, which was subsequently sprayed with a certain insecticide with the aid of a mini plastic sprayer. Each treatment had 12 replicates, which were individualized in Petri dishes (5.0-cm diameter) containing 1 ml of 2% water + agar solution. The same procedure was applied to the control (Table 1). The experiment followed a completely randomized design and was performed at 25 ± 0.2 ºC and 65% relative humidity under 12-h photoperiod.

2.2 Leaf dip method

Preliminary tests were made to establish an "all or none" response for all insecticides in the leaf dip method. Insecticides were diluted in water added with 0.01% Triton X-100 (Lima Neto et al., 2017). At least six concentrations were used in the ultimate bioassays applied to each insecticide – each treatment had three replicates. Discs (5.0-cm diameter) of *B. rapa* var. *pekinensis* leaves were immersed into the insecticide or into the control solution [water+ Triton X-100 (0.01%)] for 10s; subsequently, they were left to dry at room temperature for 1-2h (Lima Neto et al., 2017). After drying, the leaf discs were taken to Petri dishes coated with filter papers and filled with 1 ml of 2% water + agar (2%) solution. At least ten insects were placed on each treated leaf disc. The discs were kept at

25 ± 0.2 ºC and 65 ± 5% relative humidity (RH) under 12-h photoperiod. Bioassays were repeated twice. Mortality was assessed after 72-h exposure to chlorantraniliprole, pymetrozine and thiamethoxam + chlorantraniliprole because these insecticides need longer to cause death than the ones that only needed 4-h exposure to be lethal. When control mortality was >10% , the bioassys were discarded.

Tukey's test was applied to compare means recording 95% confidence level to the mortality rates recorded for different insecticides. Data were transformed through $\sqrt{x + 0.5}$. Mortality data applied to dose-response curves were subjected to Probit analysis (Finney, 1971) at P > 0.05 in the Polo-Plus 2.0 software (LeOra Software, 2005) after they were corrected for the natural mortality recorded for the control (Abbott, 1925). Toxicity ratios were calculated through the "lethal ratio test"; results were significant when confidence interval (CI) of 95% did not include the value one (Robertson et al., 2007). The insecticide presenting the lowest LC_{50} was the reference for comparisons to other insecticides.

3 Results and Discussion

M. persicae mortality due to different insecticides showed variations in comparison to the control (Table 1) after 24-h exposure through the direct spray method. Insecticides thiamethoxam + lambda-cyhalothrin, thiamethoxam, lambda-cyhalothrin and thiamethoxam + chlorantraniliprole were the most efficient in causing over 95% mortality. Pymetrozine (44.34%) and chlorantraniliprole (11.16%) showed less than 50% mortality after 48-h exposure; this result was significantly different from that of other insecticides in the Tukey test at 95% probability. Thus, based on the results, there was 100%, 100%, 100%, 99% and 97% mortality due to the exposure to thiamethoxam + lambda-cyhalothrin, thiamethoxam, lambda-cyhalothrin, thiamethoxam + chlorantraniliprole and chlorfenapyr, respectively. Only chlorantraniliprole (66.80%) was not efficient to control *M. persicae* (less than 80% mortality) within 72-h exposure.

Table 1. Mortality response from the insecticides to *M. persicae* during 24, 48 and 72 hours of exposition. Temperature 25 ± 0.2 ºC, relative humidity 65 ± 5% and 12 h photoperiod.

Tabela 1. Resposta de mortalidade dos inseticidas para *M. persicae* durante 24, 48 e 72 horas de exposição. Temperatura: 25 ± 0.2 ºC, umidade relativa: 65 ± 5% e 12 h de fotoperíodo.

Insecticides	FD *a*	N *b*	% Corrected mortality ±SE*c*		
			24h	48h	72h
Thiamethoxam + lambda-cyhalothrin	123.5 *d*	240	100.00 ± 0 a	-	-
Thiamethoxam	1125.0 *e*	240	100.00 ± 0 a	-	-
Lambda-cyhalothrin	40.0 *f*	240	100.00 ± 0 a	-	-
Thiamethoxam + chlorantraniliprole	150.0 *g*	240	97.49 ± 1.20 a	99.00 ± 0.67 a	100.00 ± 0 a
Chlorantraniliprole	20.0 *h*	240	4.65 ± 0.96 c	11.16 ± 2.87 c	66.80 ± 4.98 c
Chlorfenapyr	240.0 *i*	240	27.18 ± 1.75 b	97.32 ± 0.97 a	98.00 ± 1.05 a
Pymetrozine	250.0 *j*	240	28.04 ± 1.78 b	44.34 ± 2.94 b	84.0 ± 0.57 b
Control	-	240	0.72	1.08	10.60
V. C.*	-	-	4.92	5.69	5.15
F	-	-	1362.16	479.45	428.88
P	-	-	< 0.0001	< 0.0001	< 0.0001

Mortality means in the columns followed by the same letter did not significantly differ in the Tukey test (p<0.05). *Variation coefficient. *a* mg of a. i./l of water. *b* Number of insects. *c* Corrected mortality for Abbott (1925) and standard error. *d* Field dose to *M. persicae* in potato (*Solanum tuberosum*). *e* Field dose to *M. persicae* in lettuce (*Lactuca sativa*). *f* Field dose to *Lyriomyza huidobrensis* in potato (*Solanum tuberosum*). *g* ¼ Field dose to *Aphis gossypii* in cotton (*Gossypium hirsutum*). *h* Field dose to *Plutella xylostella* in Chinese cabbage (*Brassica rapa* var. *pekinensis*). *i* Field dose to *Plutella xylostella* in cabbage (*Brassica oleraceae* var. *capitata*). *j* Field dose to *Brevicoryne brassicae* in kale (*Brassica oleraceae* var. *acephala*.).

Response-concentration curves of all insecticides have shown good match to the Probit model (Chi-square test; P > 0.05) (Table 2). Perusal data of LC_{50} values showed that chlorfenapyr was the most toxic (LC_{50} = 0.10 mg/L) to *M. persicae* in the leaf dip method - it was followed by lambda-cyhalothrin (0.24 mg/L), pymetrozine (0.40 mg/L), thiamethoxam + lambda-cyhalothrin (0.60 mg/L), thiamethoxam + chlorantraniliprole (1.10 mg/L), thiamethoxam (4.90 mg/L) and chlorantraniliprole (9.1 mg/L). LC_{90} ranged from 0.71 to 93.80 mg/L in chlorfenapyr and chlorantraniliprole, respectively. According to Robertson et al. (2007), all toxicity ratios based on LC_{50} (TR_{50}) were significant. Therefore, chlorantraniliprole (TR_{50} = 94.46) was the least toxic insecticide to the *M. persicae* population (TR_{50} = 94.46), which was followed by thiamethoxam (TR_{50} = 51.64), thiamethoxam + chlorantraniliprole (TR_{50} = 11.11), thiamethoxam + lambda-cyhalothrin (TR_{50} = 5.82), pymetrozine (TR_{50} = 4.04) and lambda-cyhalothrin (TR_{50} = 2.50).

The present study provided toxicological evidence of some novel insecticides, such as chlorfenapyr, chlorantraniliprole and pymetrozine, to *M. persicae.* In addition, a mix of insecticides (thiamethoxam + lambda-cyhalothrin and thiamethoxam + chlorantraniliprole) seen as important tools to' insect pest management were also tested. Based on results of the direct spray method, thiamethoxam-based formulations were efficient in leading to over 95% mortality before 72-h exposure. LC_{50s} indicated that thiamethoxam and chlorantraniliprole were less toxic when they were used in separate. *M. persicae* population susceptibility was linked to life history of individuals' exposure to different insecticides in the environment. Accordingly, Nidhi et al. (2013) estimated LC_{50} equal to 48.75 mg thiamethoxam/L, whereas Lima Neto et al. (2017) showed LC_{50s} ranging from 5.00 mg to 9.00 mg thiamethoxam/L against *M. persicae*. Their study evidenced that the *M. persicae* population was not exposed to neonicotinoid. Lima Neto et al. (2017) estimated LC_{50} ranging from 5.5 mg to 10.00 mg chlorantraniliprole/L; but, based on the present study, chlorantraniliprole is not a promising insecticide against *M. persicae*. On the other hand, the efficacy of thiamethoxam against *M. persicae* was confirmed.

Table 2. Toxicity of insecticides different to *Myzus persicae*. Temperature 25 ± 0.2 ºC, relative humidity 65±5% and photoperiod 12 h.

Tabela 2. Toxicidade de diferentes inseticidas para *Myzus persicae*. Temperatura de 25 0.2 ºC, umidade relativa de 65 ± 5% e 12 h de fotoperíodo.

Insecticides	N(df)*a*	$|^2$*b*	Slope± SE*c*	LC_{50} (CI95%)*d*	LC_{90} (CI95%)*d*	TR_{50}(CI95%)*e*
Chlorfenapyr	270(5)	2.20	1.46 ± 0.30	0.10 (0.04-0.20)	0.71 (0.30-2.20)	-
Lambda-cyhalothrin	296(4)	5.52	1.90 ± 0.27	0.24 (0.09-0.36)	1.13 (0.77-2.32)	2.50 (1.08-5.09)*
Pymetrozine	240(4)	0.70	1.10 ± 0.20	0.40 (0.10-0.90)	5.10 (2.50-14.02)	4.04 (1.32-15.73)*
Thiamethoxam + lambda-cyhalothrin	290(6)	5.00	2.30 ± 0.50	0.60 (0.10-0.90)	2.01 (1.20-6.10)	5.82 (2.34-14.49)*
Thiamethoxam + chlorantraniliprole	289(5)	5.60	1.80 ± 0.31	1.10 (0.30-1.90)	5.80 (3.00-15.50)	11.11 (4.34-28.43)*
Thiamethoxam	243(6)	4.02	1.20 ± 0.30	4.90 (2.00-8.10)	58.20 (31.60-197.20)	51.64 (19.04-140.08)*
Chlorantraniliprole	250(6)	4.45	1.90 ± 0.22	9.10 (1.60-16.40)	93.80 (45.10-461.10)	94.46 (31.97-279.16)*

[a]Number of insects and degree of freedom; [b]Chi Square (P>0.05); [c]Standard Error; [d]mg of a. i./l of water and confidence intervals to 95%; [e]Toxicity ratio: ratio of estimates of the LC_{50s} of the most toxic insecticides and the insecticide least toxic (Robertson et al., 2007) and confidence intervals to 95%.

*Toxicity ratio is significant if confidence interval does not comprise the value 1.0 (Robertson et al., 2007).

Lambda-cyhalothrin efficiency against *M. persicae* was confirmed after 24-h exposure (100% mortality) through the direct spray method. Its toxicity was greater than that of the thiamethoxam + lambda-cyhalothrin mix. Ane et al. (2016) showed that lambda-cyhalothrin was efficient against *Aphis gossypii* (Glöver), *Aphis fabae* (Scopoli), *M. persicae*, *Macrosiphum euphorbiae* (Thomas) and *Chaetosiphon fragaefolii* (Cockerell) in strawberry *Fragaria ananassa* (Duch) under field conditions. According to results in the current study, the *M. persicae* colony was susceptible to thiamethoxam and lambda-cyhalothrin.

Unexpectedly, chlorfenapyr was the most toxic insecticide against *M. persicae*. Based on the results, it was efficient in the direct spray method. Chlorfenapyr was the most toxic insecticide against *M. persicae* in comparison to results in the studies by Nidhi et al. (2013) (12.98 mg chlorfenapyr/L). Field studies should be carried out in order to better assess chlorfenapyr against *M. persicae,* because this compound is mainly used as acaricide and lepidoptericide. Pymetrozine was efficient after 72-h exposure, it was the third most toxic insecticide (LC_{50} 5.1 mg/L); but it was the least toxic to *M. persicae* in comparison to results in the studies by Sadeghi et al. (2009), who estimated LC_{50} equal to 0.01 mg/L for *Acyrthosiphon pisum* (Harris).

Concentrations tested for all insecticides in the direct spray method overcame LC_{90s} estimated for the leaf dip method. Assumingly, the direct spray method caused additional intoxication in all aphid individuals due to both insecticide intake and contact. This outcome can explain the efficacy/toxicity differences between methods. The current results are relevant because LC_{90} is a parameter connected to control efficacy. According to the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA), for an insecticide to be considered effective in the field, the dose recommended by the manufacturer must cause at least 80% mortality to the insect pest population under field conditions.

All insecticides were efficient to control *M. persicae* under laboratory conditions, except for chlorantraniliprole. Susceptible *M. persicae* populations are rare because of the pressure caused by the indiscriminate use of insecticides. Therefore, the present study is important to resistance-management programs based on susceptibility monitoring/baseline and on subsequent evaluations. The herein used insecticides should be sprayed in rotation (between different groups) in order to keep populations' susceptibility and production sustainability. Developing resistance to insecticide is a matter of time, but such process should be delayed as much as possible, in pest populations.

4 Conclusions

All insecticides were effective against *M. persicae,* except for chlorantraniliprole. Chlorfenapyr, lambda-cyhalothrin, pymetrozine and thiamethoxam + lambda-cyhalothrin were the most toxic insecticides to this aphid. Thiamethoxam + chlorantraniliprole and thiamethoxam recorded intermediate toxicity to it.

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